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13. ABSTRACT ***(Maximum 200 Words)***

Although considerable progress in the understanding of prostate cancer has been made in the last few years, the basic knowledge of the biology of this disease remains elusive. The development of this cancer is related to the male sexual hormone (testosterone) but the actual mechanisms by which testosterone affects the development of this cancer is not known.

The prostate gland has at least three different types of cells that contribute to the physiology of the gland: basal, luminal and neuroendocrine cells. It is not totally clear what the relationship is between these different cell types, how testosterone affects them and which one is the target cell in prostate cancer development.

We will use new transgenic technology that allows tagging of a particular cell population and following its behavior over the life of the animal. These experiments will be performed in mice because this technology is well developed in these animals and there is a basic knowledge of the rodent prostate.

The studies proposed here will clarify some of the basic aspects of the biology of the prostate gland and the process of carcinogenesis in this organ.

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Introduction

The purpose of this research was to develop Cre/Lox technology to investigate cell lineages in the prostate gland of the mouse. The epithelium of the prostate is known to be formed by at least three cellular subtypes. The luminal cells have a secretory function and are the major component of the prostate epithelium. Between the layer of luminal cells and the basement membrane is another compartment formed by basal cells. This third cellular type is neuroendocrine cells, a minor population of cells interspersed among the luminal cells (Wang et al., 2001).

The luminal cells are believed to be differentiated cells derived from the basal cells. Although they have proliferative activity, this is limited to a few divisions before they become apoptotic. They express androgen receptor and have secretory characteristics under the control of androgens. They express specific markers, e.g. Keratin 8 and 18. The basal cells are very small cells expressing Keratins 5 and 14. They are thought to be the proliferative compartment and it is also believed that at least a subpopulation of the basal cells may be stem cells. Neuroendocrine cells are a minor component probably involved in intercellular signaling. They are also important because many tumors express neuroendocrine markers suggesting that either some tumors derive from this particular cell type or differentiate into this phenotype (Hsieh et al., 1992; El-Alfy M et al., 2000; Wang et al., 2001; Bonkhoff, 2001).

This paradigm of tissue kinetics in the prostate is not supported by hard core evidence and has been questioned by several investigators. Furthermore, although the luminal cells appear to be terminal cells, tumors derived from the prostate have all the characteristics of this cell type. Furthermore, no tumors with the phenotypic characteristic of basal cells have been described and only non-neoplastic hyperplasia has been described in this subpopulation (Foster and Bostwick, 1998).

The goal of this project is to develop a system that allows permanent labeling of the different cell subpopulations using Cre/Lox technology (Saue, 1998). The Cre gene was put under the control of Keratin 5 and 14 promoters (markers of basal cells) as well as the probasin promoter (marker for luminal cells). We have also obtained Rosa 26-β gal mice. Rosa 26 is a universal promoter, in this case driving a β gal gene preceded by a floxed stop sequence (Zambrowics et al., 1997). Crossing these animals with Cre expressing mice lead to recombination for the Rosa 26 minigene with loss of the stop sequence and expression of the β gal message. Since keratin markers are lost or gained as cells differentiate, by using the keratin promoters driving the CRE gene, we can permanently label cells in a given compartment and these cells will remain labeled regardless of their differentiation status.

Once these animals are obtained, the goal of the project will be to track the cell lineages in the prostate under different hormonal conditions. Also by crossing these animals with two well established models of prostate cancer, we will investigate the cells of origin of the tumors.

BODY

Animals:

The first stage of the project was to obtain or developed all the transgenic mice to be used in the project. This is the list of animals and the current status for each of them.

K5-Cre:

These animals have been developed using our K5 cassette, originally obtained from Dr. Jose Jorcano (Ciemat, Madrid Spain) are established in our facility and used routinely in our transgenic experiments (Robles et al., 1996; Rodriguez Puebla et al., 2000). Crosses with Rosa 26 mice have already been performed (see below).

K14-Cre

These animals have been developed in collaboration with Dr. DiGiovanni and the colony was established at our facility. Crosses with Rosa 26 mice have already been performed (see below).

Rosa-26

These animals were obtained from our Core Facility in Houston with permission of Dr. P. Soriano (Zambrowicz BP et al., 1997) and have already been used in crosses with K5-Cre and K14-Cre.

Probasin Cre

These animals were recently received from the University of Southern California (Wu et al., 2000). The mice were requested before the starting date of the grant but were only recently received due to problems with the MTA. Lawyers in our institution questioned the language of the MTA submitted by USC. Changes introduced by our lawyers needed to be approved not only by USC but also by pertinent officers of the University of Manitoba as that institution holds the original patent on some of the reagents used to develop these mice. Two males were received on April 16, 2002 and are being backcrossed with B6/D2, the original background, to stabilize the colony.

K5-CreERT^2

These mice are similar to K5-Cre but the Cre gene has been replaced by a fusion protein between Cre and a mutated estrogen receptor. These primers were developed at our facility using reagents from P. Chambon's laboratory (Indra et al., 1999).

K14-CrePR

These animals are identical to K5-CreER^{T2} but the transgene is driven by the K14 promoter. They have been obtained from P. Chambon's laboratory (Indra et al., 1999).

K5 IGF

These mice have been developed at SPRD and are available in our colony. They were described in the original submission of the grant (DiGiovanni et al., 2000).

TRAMP Mice
This is a metastatic model of prostate cancer generated by transgene expression of a T antigen under the control of the probasin promoter. These animals have been received from Dr. NM Greenberg (Gingrich1996) and the colony is stabilized in our animal facility.

Key Research Accomplishments

The experiments took longer than expected because of delays in obtaining and developing the mice. We underestimated the time required to obtain the animals and, in particular, to stabilize the colony and breed enough mice to perform the experiments. However, some interesting results have been obtained. The first crosses with K5-Cre gave us a sense of the problems we will face with these experiments and we will be able to control these problems in advance. Also, in preparation for the experiments of carcinogenesis we have continued our experiments, better defining the K5-IGF model and found some answers to the hypothesis stated in this grant. The experimental results are described below.

K5-Cre x Rosa 26 Crosses
K5-Cre mice were crossed with Rosa 26 mice and 6 double transgenic mice were obtained. The transgenes are detected by PCR. In the case of K5-Cre we used the strategy used routinely in our laboratory with primers for the Globin intron (Rodriguez Puebla et al. 2000). In the case of the Rosa 26 we used primers provided by Dr. Soriano (Zambrowicz BP et al., 1997).

Transgenic mice were sacrificed at week 6, the prostates were removed and frozen sections from dorsal and ventral prostates were obtained. The presence of β gal was demonstrated with X gal using a commercial kit (Specialty Media, Phillisburg, NJ).

Results were as expected. The luminal epithelium was marked. As discussed in the application, the accepted paradigm of prostate development is that all the epithelial cells derived from a common embryonary precursor which expresses Keratin 5 (Hayward and Cunha, 2000). The experiment confirms that paradigm. As suggested by one of the reviewers of the grant and our own assessment, the simple K5-Cre transgenic mice will not be able to distinguish populations of the adult prostate because all the cells would already be positive. That is the reason we need to use the K5-inducible Cre mice in order to be able to control the time of recombination. However, it was important to confirm the current paradigm with the new technology because the whole interpretation of these experiments is based on the fact that all the cell populations in the prostate derive from a common precursor.

In addition to confirming the current paradigm with a more definitive strategy, this experiment also was used to learn more about the system, look for potential problems and develop alternative strategies. One problem that we need to deal with is the localization of β in the basal cells. Looking at the X gal preparations it was evident that it will not be easy to show that label unequivocally in the basal cells. In addition, we are concerned that the signal with the Rosa 26 promoter is not very high in the prostate cells. The Rosa promoter may not be a strong promoter in this cellular type and therefore we need to develop more sensitive methods for the detection of β gal. Approaches based on fluorescent substrates have been developed. This methodology will allow the detection of β gal with the confocal microscope (Cejkova J et al., 1999; Geigher et al., 1992). We expect that this methodology will solve both problems.

The second interesting result came from the study of the K5-IGF model of carcinogenesis. This model was developed in collaboration with Dr. DiGiovanni and the phenotype of these animals as well as the preliminary description of the animals has been previously published (DiGiovanni et al., 2000). We propose in this grant to use these mice for the identification of the cell of origin of the tumor. So, we have proceeded to a more detailed description of the pathology of these animals and in preparation for the experiments of this proposal we made a careful analysis of the keratins expressed by the different normal and neoplastic cells in these mice. We found several interesting results. First, there is an expansion of the basal compartment (K5 positive cells) in the preneoplastic process and, more interesting, the lesion, whether preneoplastic or neoplastic, presents an accumulation of cells expressing markers for both luminal and basal cells. The existence of these "intermediate" cells has been suggested by other laboratories and evidence supporting their existence has been presented (Hudson et al., 2000). But this is, to our knowledge, the first direct and unequivocal evidence for the existence of this intermediate cell population. It is worth mentioning that these cells have been postulated to be the primary target of the carcinogenesis process.

Reportable Outcomes

None

Conclusions

It is too early at this point to obtain relevant scientific data from those experiments involving extensive breeding and crossing genetically engineered mice. As discussed above these experiments are taking a long time and we underestimated the time frame for the experiments.

However, there was a major scientific accomplishment in this period related to the identification of an intermediate cell population. The cellular mechanisms involved in the early stages of prostate cancer development are very poorly understood. A major contributing factor for this lack of knowledge is the limited understanding of the cell lineages in the prostate epithelium and in consequence the uncertainty of the cell populations involved in those early carcinogenesis events. The unequivocal demonstration of an intermediate cell population expressing markers of basal and luminal cells in our K5-IGF model opens the possibility of new studies to clarify these problems, including some of the experiments proposed in our grant.

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